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Microbiological and Pharmacological Behavior of 7-Chlorolincomycin

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Replacement of the 7-(R) hydroxyl group of lincomycin by a 7-chloro-substituent produced a compound with greater in vitro activity than the parent. Laboratory studies of this compound showed it to be highly active against all of the following strains of gram-positive organisms examined, including penicillinase- and nonpenicillinase-producing staphylococci, *Diplococcus pneumoniae*, *Streptococcus viridans* and *Streptococcus pyogenes*. The enterococci, as well as all the gram-negative organisms tested, with the exception of some strains of *Haemophilus*, were uniformly insensitive to this agent. The activity of 7-chlorolincomycin was not affected by serum or inoculum size. Resistance developed in a slow stepwise pattern. Peak levels of approximately 2 μg/ml were achieved in the serum of volunteers after ingestion of 150 mg either in the fasting state or after a meal. No untoward effects were noted. The antibiotic appears to be of potential value in the treatment of infections due to gram-positive organisms, with the exception of enterococcus.

Lincomycin, an antibiotic produced by *Streptomyces lincolnensis*, is an effective drug for the treatment of infections caused by a number of gram-positive bacteria (5). Replacement of the 7-(R) hydroxyl group by a 7-chloro substituent in the 7-(*S*) configuration produces the derivative, 7-chlorolincomycin (Fig. 1). This agent is said to possess a greater degree of antibacterial activity in vitro than the parent drug (2, 6; C. Lewis, K. F. Stern, and D. T. Mason, Antimicrobial Agents and Chemotherapy, *in press*.). The substituted compound has been reported to be active in vitro against most gram-positive organisms, including *Streptococcus faecalis* (6). The purposes of this study were to investigate the antibacterial activity of 7-chlorolincomycin in vitro, to study naturally occurring and artificially induced resistance to the drug, and to examine its absorption and excretion under various conditions in man.

MATERIALS AND METHODS

Microbiological studies. The minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations of the antibiotic were determined in the following manner. The medium employed for all of these studies was Brain Heart Infusion broth (Difco). Standard serial double-dilution procedures were employed. Organisms examined were all freshly isolated from hospitalized patients. Overnight culture of each strain prepared as a 10^{-5} dilution served as the inoculum. Sterile 7-chlorolincomycin powder was dissolved in 0.2 M phosphate buffer (*p*H 7.1) and stored at −30 C until used. Tubes containing broth and various dilutions of the antibiotic were inoculated with 0.5 ml of bacteria, incubated at 37 C for 18 hr, and then examined for visible evidence of growth. The lowest drug concentration which inhibited growth was the MIC. All clear tubes were subcultured on blood-agar which was incubated at 37 C for 18 hr. The lowest drug concentration from which subcultures were sterile was considered the MBC of the compound.

The effect of inoculum size on the MIC and MBC of 7-chlorolincomycin was evaluated with seven strains of *Staphylococcus aureus* in doses of 1 to 2 × 10^2, 10^4, and 10^6 organisms. To determine the effect of protein binding on antibacterial activity, the antibiotic was serially diluted in broth containing 20, 50, and 75% human serum, after which the standard-size inoculum was introduced, the tubes were incubated at 37 C overnight, and the presence or absence of growth was noted.

Four strains of *S. aureus* were serially transferred in broth containing twofold increases in concentrations of 7-chlorolincomycin. The tube with the largest quantity of antibiotic in which bacterial growth was detected served as the source of the inoculum for the next transfer. The experiment was terminated when the organisms failed to be inhibited by a concentration of drug equal to approximately 30 times the initial MIC.

Studies were carried out to determine if diffusible substances produced by resistant gram-negative organisms inactivated or blocked the effect of the drug in vitro. Heart Infusion Agar (Difco) plates containing serial twofold dilutions of 7-chlorolincomycin (0.5 to 0.015 μg/ml) were prepared. They were then inocu-

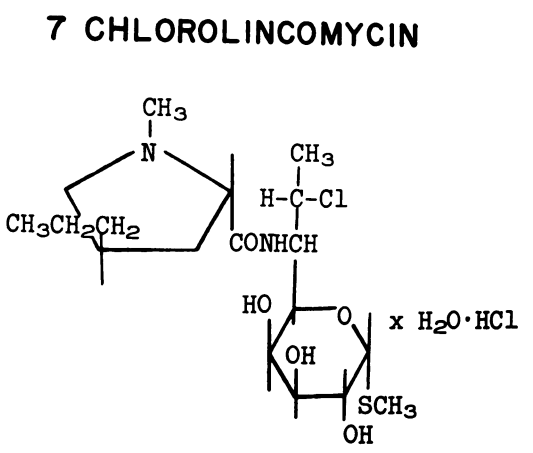

FIG. 1. *Structure of 7-chlorolincomycin.*

lated with five strains of *S. aureus* (an overnight culture) diluted to 10^{-3}. Seitz filtrates of cultures of drug-resistant strains of *Pseudomonas aeruginosa, Escherichia coli, Alcaligenes faecalis, Klebsiella aerogenes,* and *Serratia marcescens* were layered over the surface of the agar. Controls consisted of agar plate cultures of the staphylococci to which material from the gram-negative organisms was not added. The MIC in the experimental and control cultures was determined after incubation at 37 C for 18 hr. Another method that was used involved the inoculation of a 7-chlorolincomycin-sensitive strain of *Staphylococcus aureus* into tubes of melted agar containing various concentrations of the drug, and plates were poured. After the agar was hard, a loopful of an overnight culture of drug-resistant strains of *P. aeruginosa, E. coli, A. faecalis, K. aerogenes,* and *Serratia marcesens* were streaked on the surface. After incubation at 37 C for 18 hr, each culture was examined for the presence of staphylococcal growth in the area of colonies of the gram-negative bacilli. (3)

Pharmacological studies. Ten healthy men, aged 21 to 35 years, were the subjects for this study. Blood samples for three of the experiments were obtained just before and at 0.5, 1, 2, 4, 6, and 8 hr after the drug was administered. Four schedules were examined (Table 3). In the first schedule, subjects fasted for 8 hr before and 2 hr after they were given a single dose of 150 mg of 7-chlorolincomycin orally. The second procedure was the same as in the first schedule except that the antibiotic was taken 0.5 hr after a meal. In the third experiment, subjects were fasting. They received 0.5 g of probenecid in the fasting state. This was followed 1 hr later by the oral administration of 150 mg of the antibiotic. For the fourth experiment, a dose of 7-chlorolincomycin (150 mg) was administered in the fasting state. The same quantity was given 8 and 16 hr later. Blood was drawn after 1, 9, and 24 hr. Urine was collected for the first 8 hr and then for 8 to 24 hr after drug ingestion. All of the serum and urine samples were stored at −30 C until they were assayed for concentration of 7-chlorolincomycin, by the use of the cup plate method with *Staphylococcus* 209P as the test organism (4).

The following additional laboratory studies were carried out before and after the treatment period: complete blood count, urinalysis, blood urea nitrogen (BUN), serum creatinine, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), bilirubin, and alkaline phosphatase.

RESULTS

Microbiological studies. The results of studies of the in vitro susceptibility of a number of bacterial species to 7-chlorolincomycin are presented in Tables 1 and 2. All of the strains of *S. aureus* (penicillin-sensitive and resistant), *Streptococcus pyogenes, Streptococcus viridans,* and *Diplococcus pneumoniae* were inhibited by concentrations of the drug ranging from 0.015 to 0.3 μg/ml. The enterococci were much less sensitive; their growth was suppressed only in the presence of 12.5 to 50 μg/ml of the antibiotic. *E. coli, P. aeruginosa, K. aerogenes,* indole-negative and positive strains of *Proteus, Salmonella, Serratia marcescens, Shigella,* and *A. faecalis* were not inhibited by less than 1 μg/ml; some were not suppressed by concentrations as high as 500 μg/ml. The MIC and MBC of all the pneumococcal and streptococcal strains examined were approximately equal. The same was true for 50% of the staphylococci studied; the MBC for the others was, at most, fourfold higher than the MIC.

Attempts to induce drug-resistance by daily serial subcultures of four strains of *Staphylococcus aureus* in 7-chlorolincomycin were made. Each strain had an initial MIC of 0.07 μg/ml. Significant loss of sensitivity of the antibiotic appeared only after the 9th or 10th transfer with three strains and after the 5th transfer with one;

TABLE 1. *In vitro activity of 7-chlorolincomycin on gram-positive cocci*[a]

Concn of drug (μg/ml)	No. of strains inhibited							
	Streptococcus pyogenes		*Streptomyces viridans*		*D. pneumoniae*		*Staphylococcus aureus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
0.01	6	6	2	2	5	3	8	1
0.05	2	2	3	3		2	17	5
0.12							18	26
0.30	3	3						7
0.60								2
1.20								2
3.00								

[a] Of the enterococci, two strains were inhibited at concentrations (MIC and MBC) of 12 μg/ml and five strains were inhibited at concentrations (MIC and MBC) of 50 μg/ml.

TABLE 2. *In vitro activity of 7-chlorolincomycin on gram-negative bacilli*

Concn of drug (μg/ml)	No. of strains inhibited															
	Salmonella		Shigella		Proteus		Aerobacter		Pseudomonas		Paracolon		Serratia		Haemophilus	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<1.2															3	
1.2																3
3.0															2	
6.0													1			
12	1												2			
25	1	1	3				3				1		1	1		2
50					5	5			1	1				1		
100		1		3				3				1	15	17		

the MIC after subculture was 2.5 μg/ml in each case.

Inoculum size played no significant role in determining the in vitro effects of 7-chlorolincomycin. The MIC for seven strains of *S. aureus* was identical when inocula containing 10^2, 10^4, or 10^6 organisms per ml were employed.

Studies of the effects of various concentrations of human serum revealed no effect on the activity of the drug. The MIC for five strains of *S. aureus* was identical when these organisms were grown in drug-broth containing 20, 50, or 75% serum.

The antimicrobial activity of 7-chlorolincomycin was not inhibited by exposure to gram-negative bacteria or to filtrates of cultures of these species.

Pharmacological studies. At 0.5 hr after a single dose of 150 mg of drug was given orally in the fasting state, the average serum level was 1.75 μg/ml. Only trace amounts (< 0.1 μg/ml) were detected in two subjects at this time. Average serum concentration present at 1 hr was 1.86 μg/ml, with a range from 1.0 to 3.2 μg/ml. At 2 hr, the average levels were 1.53 μg/ml; (range 1.2 to 2 μg/ml). The average serum concentration after 4 hr was 1.18 μg/ml; only trace amounts were found in three cases; levels of 0.7, 1.3, 1.7, and 2.5 μg/ml were detected in the others. At 6 hr after the antibiotic was administered, only traces of it were demonstrated in the blood of six of the subjects. After 8 hr, only one individual had a significant quantity of antimicrobial activity (0.75 μg/ml) in the blood.

Nine subjects were given a single dose of 150 mg of 7-chlorolincomycin after breakfast. Seven of them revealed only traces of antibiotic in the serum 0.5 hr after they were treated. For all specimens at 1 hr, the average was 2.04 μg/ml. The average serum concentration after 2 hr was 1.87 μg/ml; the range was 0.2 to 4 μg/ml. Only two of the individuals were found to have detectable serum antibacterial activity (1.5 μg/ml) after 6 hr; only traces were present in all of the subjects 8 hr after they had been treated.

Volunteers in the fasting state (10) were given 500 mg of probenecid 1 hr before ingestion of 150 mg of 7-chlorolincomycin. After 0.5 hr, only traces of drug were detected in the serum of four individuals; in the others, the values were 1.0 to 2.1 μg/ml, the average for the group being 1.57 μg/ml. Determinations of blood levels 1 hr after ingestion revealed the average level was 2.04 μg/ml. Two hours after the dose the average quantity present in the circulation was 1.28 μg/ml; individual levels ranged from 0.3 to 2.8 μg/ml. After 4 hr only traces of antimicrobial activity could be demonstrated in four persons; in four, the concentration was approximately 1.0 μg/ml;

TABLE 3. *Average serum concentrations of 7-chlorolincomycin*

Time after dose	Expt[a]			
	A	B	C	D
hr				
0				
0.5	1.75	Tr	1.57	
1	1.86	2.04	2.04	1.98
2	1.53	1.87	1.28	
4	1.18	1.0	1.13	
6	0.54	Tr	Tr	
8	Tr	Tr	Tr	
9				2.6
24				Tr

[a] Trace (Tr) < 0.1 μg/ml; (A) 150 mg of drug in fasting state, 10 subjects; (B) 150 mg of drug after a meal, 9 subjects; (C) probenecid followed by 150 mg of drug, 10 subjects; (D) 150 mg at once and at 8 and 16 hours later, 9 subjects. Values are expressed in micrograms of drug per milliliter.

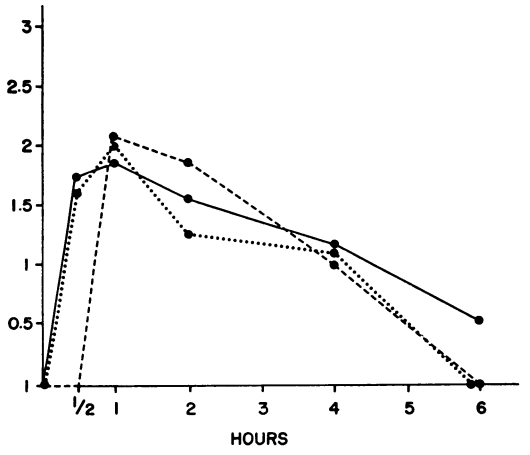

FIG. 2. *Average serum concentration of 7-chlorolincomycin (μg/ml) after 150 mg in the fasting state (solid line), with 150 mg after a meal (broken line) and with 150 mg administered after a 500-mg dose of probenecid (dotted line).*

TABLE 4. *Urinary excretion of 7-chlorolincomycin on the first schedule*[a]

Subject no.	Amt of drug in urine (mg)	
	0 to 8 hr	9 to 24 hr
1	2.7	2.3
2	2.8	0.3
3	2.2	
4	2.7	1.5
5	4.9	0.8
6	0.33	1.4
7	3	0.8
8	1.9	0.9
9	2.4	0.3

[a] Total output over 24 hr was 3.6 mg. The average amount for 0 to 8 hr was 2.55 mg and for 9 to 24 hr was 1.04 mg.

in the other two, it was 2.4 μg/ml. Only two individuals had significant amounts of antibiotic in the blood (1.5 μg/ml) after 6 hr. None was detected 8 hr after the drug was administered (Table 3 and Fig. 2).

In our test with repeated oral administration of drug, nine subjects received 150 mg of 7-chlorolincomycin orally at the beginning of the experiment and at 8 and 16 hr later. At 1 hr after treatment was started, the average serum concentration was 1.98 μg/ml. The average blood level 8 hr later was 2.61 μg/ml. Significant quantities of drug could not be demonstrated in the blood of any of the subjects 24 hr after treatment was started. (Table 3)

The average quantity of 7-chlorolincomycin excreted in the urine in the first 8 hr after a single oral dose of 150 mg given in the fasting state was 2.55 mg, with a range of 0.3 to 4.95 mg; most individuals excreted 2 to 3 mg over this period. During the next 16 hr, an average of 1.04 mg of the antibiotic was present in the urine (range, 0.3 to 1.5 mg; Table 4). The average total excretion for the 24-hr period after the first dose was given was between 3.6 (fasting) and 8 mg (after a meal), or 2.4 to 5.3% of the quantity administered.

Complete blood counts, urinalyses, SGOT, SGPT, serum bilirubin, alkaline phosphatase, BUN, and serum creatinine remained within normal limits during and after completion of the studies.

No untoward effects were observed in any of the subjects.

DISCUSSION

Studies described in this paper indicated that 7-chlorolincomycin was highly active in vitro against the strains of *D. pneumoniae*, *Streptococcus viridans*, *S. pyogenes* and penicillin-G sensitive and resistant *Staphylococcus aureus* that were examined. All of these organisms were inhibited by concentrations of 0.015 to 0.3 μg/ml of the drug. Enterococci were found to be highly resistant to this antibiotic, their growth being suppressed only in the presence of 12.5 to 50 μg/ml. These data and studies by other investigators, (2; C. Lewis, K. F. Stern, and D. T. Mason. Antimicrobial Agents and Chemotherapy, *in press.*) are not in agreement with those of Magerlein (6) who reported that this type of streptococcus was sensitive to 0.16 μg/ml of 7-chlorolincomycin. All of the gram-negative bacteria studied except some *Haemophilus* strains were highly resistant to this antimicrobial agent. Attempts to demonstrate the production of drug-blocking or inactivating substances by these species were unsuccessful. Eriksen and Hansen (1), however, have indicated that conflicting results regarding β-lactamase activity in the filtrates of *S. aureus* after passage through a Seitz filter may be due to nonspecific variable adsorption to the filter surface. The possibility exists that extracellular enzymes produced by resistant gram-negative bacteria may also be adsorbed into filter surfaces. Repeated exposure of *S. aureus* to 7-chlorolincomycin revealed a slow stepwise increase in resistance. Size of inoculum appeared to have no effect on the in vitro activity of the drug. The degree of antibacterial activity was unaffected by high concentrations of serum.

Pharmacologic studies revealed that peak serum levels of about 1.9 μg/ml were present 1 hr after a single oral dose of 150 mg of 7-chloro-

lincomycin. This fell gradually to less than 1 μg/ml over a 4-hr period. Dosing after a meal or probenecid administration appeared to have no effect on peak blood levels, the average quantity demonstrable after 1 hr being 2 μg/ml. These concentrations are about 10 times greater than those required to suppress the growth of all of the strains of staphylococci, pneumococci, *Streptomyces viridans* and *Streptococcus pyogenes* studied, and are higher than the MBC for all of these organisms. Approximately 2.4% of the drug was excreted in the urine during a 24-hr period. The administration of 3 doses of 150 mg each at 8-hr intervals resulted in the presence of only trace amounts of antibacterial activity in the serum 24 hr later. This suggests that accumulation of 7-chlorolincomycin in the circulation does not occur. Clinical and laboratory studies revealed no untoward effects in the subjects treated.

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